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ANALYSIS OF THE FOLIAR ANATOMY OF SIX DISTINCT VARIETIES OF PIPER BETLE L

Vikas Kumar
Ph.D., Research Scholar, Department of Life Science (Botany), Singhania University, Jhunjhunu, Rajasthan (NET/JRF Qualified)

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Abstract:

The current study focused on examining specific cultivars of Piper betle L. in order to gain insights into the anatomical structure and tissue composition of the stem, leaves, and petiole. The achievement was attained through the utilisation of fundamental anatomical techniques, including free hand and microtome sectioning. The selected varieties include HY1, HY2, JB, KB, LV, and SG. The primary components of the current investigation encompassed macro morphology, anatomy, and histochemistry. The primary objective of the study was to investigate the internal composition of leaves and petioles in order to gain insights into the functions of specialised cells, such as enlarged hypodermal cells and mucilage cavities. Specifically, the study aimed to understand how these cellular features contribute to moisture retention and subsequently enhance the longevity of harvested leaves.

Key Words: Anatomical Study, Piper Betle, Hypodermis, Secretary Cell, Mucilage Cavity

Introduction:

Piper betle Linn., a dioecious plant belonging to the Piperaceae family, is an annual creeper that exhibits climbing behaviour through numerous small adventitious rootlets. It typically reaches a height of approximately one metre and is commonly cultivated in regions characterised by higher temperatures and humidity levels. This particular species is commonly found in humid forest environments and is cultivated in various regions including India, Vietnam, and China in South-East Asia. The species is distributed in various regions of India, including Uttar Pradesh, Bihar, Bengal, Orissa, Tamilnadu, Andhra Pradesh, and Karnataka. In the state of Tamil Nadu, three predominant varieties of P. betle leaves are commonly available, namely Sirugamani, Karpoori, and Vellaikodi. This substance is utilised in various decoctions and has been found effective in treating wounds, burns, impetigo, furunculosis, eczema, lymphangitis, and as a beneficial stomatic agent. The Kammaru leaf, a variety of P. betle, possesses a significant quantity of juice that exhibits healing properties for conditions such as pharyngitis, abdominal pain, and swelling. In general, betel leaf is known to have potential benefits in treating urticaria. According to Ayurvedic medicine, it is believed to restore the balance between the three fundamental bodily humours, namely Vata, Pitta, and Kapha, which may be disrupted. The roots and fruits have been widely recognised for their therapeutic properties in the treatment of malaria and asthma (Bhattacharya et al., 2007).

The P. betle leaf has been reported to contain Piperol-A, Piperol-B, and methyl piper betlol, which have been isolated according to a study by Chopra et al. in 1956. The betel leaves contain a variety of components including starch, sugars, diastases, and an essential oil. The essential oil is composed of terpinen-4-ol, safrole, allyl pyrocatechol monoacetate, eugenol, eugenyl acetate, hydroxyl chavicol, eugenol, piper betol, and other key components such as cadinene carvacrol, allyl catechol, chavicol, p-cymene, caryophyllene, chavibetol, cineole, estragol, and more. The leaves underwent a phytochemical analysis, which indicated the presence of alkaloids, tannins, carbohydrates, amino acids, and steroidal components. The primary constituent of the leaves is a volatile oil known as Betle oil, which is sourced from various countries. This oil contains two phenols, namely betle phenol (Chavibetol) and Chavicol. Codeine has also been discovered.

The mixture of P. betle leaves combined with salt and hot water can be utilised for the treatment of filariasis. A recommended treatment for obesity involves the consumption of a mixture consisting of one P. betle leaf combined with Piper nigrum for a duration of two months.

The combination of P. betle juice and honey is considered beneficial for the treatment of coughs, dyspnea, and indigestion in paediatric patients. The application of oil on the breasts of lactating women using leaves of P. betle is believed to have potential benefits in promoting milk secretion. It is advisable to utilise a localised application for the treatment of inflammatory swelling conditions, such as orchitis, arthritis, and mastitis. In the case of both children and elderly individuals, a traditional remedy involves combining leaves with mustard oil, heating the mixture, and applying it to the chest. This treatment is commonly used to alleviate symptoms of coughing and difficulty breathing. This product effectively addresses issues related to unpleasant breath, body odour, and tooth decay prevention. This product is designed to prevent and treat vaginal discharge, as well as alleviate vaginal itching. Please provide guidance on how to manage a nosebleed. The product is enriched with essential vitamins including thiamine, niacin, riboflavin, and carotene. In India, leaves are utilised for the treatment of various medical conditions such as eczema, lymphangitis, asthma, and rheumatism. A poultice made from leaves is applied to cuts and wounds. The combination of roots and black pepper has been

traditionally believed to have potential effects on female fertility. The oil is utilised for alleviating irritation in the throat, larynx, and bronchi, as well as for gargling and inhalation in cases of diphtheria. The juice extracted from leaves is utilised for its stomachic and febrifuge properties. According to Vandana and Shalini (2014).

Material and Methods:

The current study was conducted on six distinct varieties of Piper betle, namely Hybrid I (HY1), Hybrid 2 (HY2), Jaipur Bangla (JB), Karpoori (KAR), Tenkasi Variety (TV), and Sirugamani (SG). The plant cuttings were obtained from the Sugarcane Research Station located in Sirugamani, Tamil Nadu, India.

The cuttings were cultivated in the nursery of the department without the use of organic or inorganic fertilisers, while being adequately irrigated. For anatomical investigations, a uniform-sized segment from the seventh internode (located in the middle of the plant) of each variety was collected and cut into small segments. Fresh transverse sections of the leaf, stem, and petiole were obtained and examined using a compound light microscope (Nikon Alphaphot). The detection of starch and oil was carried out using specific stains and reagents, following the established protocols outlined by Krishnamurthy (1988), Harris and Oparka (1994), and Dayanandan et al. (1996).

Microtome sections were obtained using the conventional paraffin embedding technique as outlined by Johansen (1940). The study utilised six samples of stem, leaf, and petiole, each obtained from six distinct varieties. Small pieces (5 x 5 x 10 mm) of the stem, leaf, and petiole were freshly cut and subsequently immersed in a formalin acetic acid-alcohol (FAA) solution for a duration of 48 hours to facilitate fixation. Subsequently, the materials underwent dehydration using a series of alcohol solutions, specifically 25%, 50%, 75%, and 100%. Subsequently, the materials underwent a xylol series consisting of solutions with concentrations of 25%, 50%, 75%, and 100%. The materials were then allowed to rest for a duration of 4 to 6 hours. The materials used consisted of xylol with a purity of 100%, and the process of wax infiltration was initiated. The specimens underwent infiltration with paraffin wax. The paper boats were utilised for the purpose of casting the blocks, and they were stored in a refrigeration unit before undergoing microtome sectioning.

Transverse sections were obtained with a thickness of 18 µm and affixed onto slides using Haupt's adhesive. The sections were immersed in a 4% formalin solution for proper fixation. The sections were stained using Toludine blue 'O' as described by Feder and O'Brien in 1968. Following the staining process, the slides were allowed to air dry. Subsequently, the slides were dewaxed by passing them through two successive changes of pure xylene. Finally, the slides were mounted using DPX to ensure their permanence.

Results:

The primary objective of the current study was to expand the existing knowledge on the anatomical characteristics of several specific cultivars of P. betle. Additionally, the study aimed to gain insights into the functions and roles of different tissue components present in the betle leaf, as outlined in Table 1. The varieties chosen for these investigations are HY1 (Hybrid Variety 1 - male), HY2 (Hybrid Variety 2 - female), Jaipur Bangla (JB), Karpoori (KP), Tenkasi Variety (TV), and Sirugumani (SG) (Figures 1a - 1f).

In all of the varieties that have been examined, the upper epidermis is composed of a single layer of cells with thin walls, and it is enclosed by a thin layer of the cuticle (see Figure 2a). The shape of the epidermal cells is isodiametric; however, there is variation in size among the different varieties that have been studied (see Figures 2b and 2c). Trichomes are located directly above the epidermis and are characterised by being uniseriate and unicellular in nature. The trichome is characterised by a basal stalk cell and typically exhibits a conical or oval shape. In the HY2 and Sirugumani varieties, it is observed that the trichomes consist of three cells. The hypodermal cells are characterised by their larger size in comparison to the epidermal cells, and they are organised into two distinct layers. The cells exhibit a cuboidal shape with minor folding, measuring approximately 38 µm in length across all variations. The width ranges from 52 to 78 µm. The presence of large-sized hypodermal cells was observed in the Karpoori, HY2, JB, and Sirugamani samples (Figures 2b & 2c). The cells exhibit a high degree of compactness, lacking any intercellular spaces. The Hypodermis also exhibits secretory cells and mucilage canals (Figure 2d). The upper epidermis lacks stomata entirely. Similar to the upper epidermis, the lower epidermis is also composed of a single layer of cells. These cells have a similar length to the upper epidermal cells, but their breadth is slightly reduced, resulting in an oblong shape (Figure 2c). The stomata exhibit a uniform distribution on the lower surface. The stomata present in this context exhibit the anomocytic type. The stomatal frequency differs among various cultivars as follows: HY1 with 128 stomata per square millimetre, HY2 with 138 stomata per square millimetre, JB with 116 stomata per square millimetre, Karpoori with 124 stomata per square millimetre, LV with 128 stomata per square millimetre, and Sirugamani with 137 stomata per square millimetre. The lower hypodermal cells consist of a single layer, except in the areas between two vascular bundles where they form a double layer (Figure 2a). In the various types examined, two distinct types of trichomes were observed: unicellular and uniseriate multicellular.

The mesophyll is composed of palisade cells and spongy parenchyma, giving it a bifacial structure (Figure 2a). It can be observed that the palisade layer is consistently present within a single stratum across the majority of the leaf. Secretory cells are also present interspersed among the palisade cells. The spongy tissue consists of cells that are irregularly shaped. The mesophyll tissue is characterised by the presence of calcium

oxalate and fine sand crystals. The crystals were easily visible when observed under a polarised microscope (Figure 2e). Previous studies have reported the occurrence of granular crystals in the leaf lamina, particularly in the spongy parenchyma, of P. betle. Within the midrib region, there exists a solitary, substantial vascular bundle that takes on a bowl-like shape (refer to Figure 2a). The hypodermis in this particular region is replaced by a cluster of collenchyma cells. There is a single large mucilage canal measuring approximately 100-150/m located above the vascular bundle (Figure 2e). The presence of thick-walled xylem elements is observed, with the phloem located beneath the xylem.

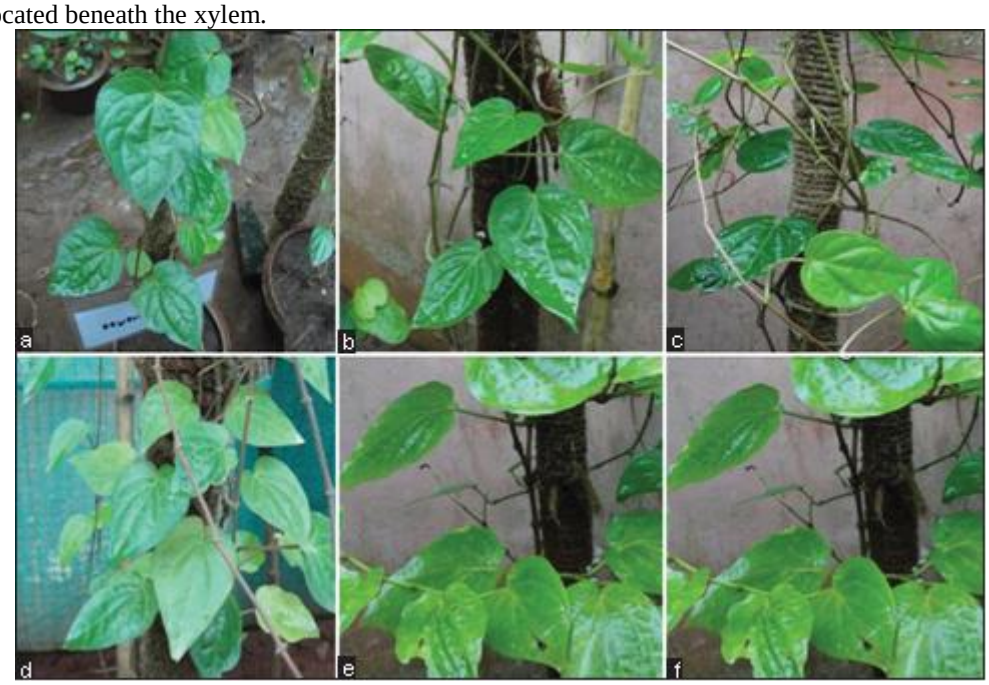

Figure 1: Habit of *P. betle* cultivar a) Hybrid I (HY1), b) Hybrid II (HY2), c) Jaipur Bangla (JB), d) Karpuri (KAR), e) Tenkasi variety (TV) and (f) Sirugamani (SG)

The hypodermal layer of the betel leaves, characterised by lateral wall folding, has been identified as the most prominent feature by Chibber (1913), Metcalfe & Chalk (1950), and Raman et al. (2012) (see Figure 2e). This suggests the presence of a mechanism to decrease the dimensions of the cell lumina of the hypodermal aqueous cells, if required. According to Metcalfe and Chalk (1950), these cells are significantly larger than epidermal cells, measuring approximately three times their size when fully turgid. Similar features were observed in the hypodermal cells on both the upper and lower surfaces of the selected varieties. According to Chibber's report in 1913, it was observed that the hypodermal cells exhibit a significant degree of silicification. This characteristic is believed to serve the purpose of preventing the collapse of the aqueous hypodermis. Additionally, the unicellular trichomes exhibit structural similarities to hydathodes and have the ability to absorb water from the external environment when it is both necessary and accessible (Raman et al., 2012). The basal cell of the trichome facilitates the process of water entry into the leaves.

The ten cultivars of P. betle exhibit certain structural similarities. In all the varieties of P. betle that were examined, it was observed that there is a four-layered upper epidermis and a two-layered lower epidermis. Crystals and oil reserves were discovered within the epidermal cells. Certain varieties exhibited a higher frequency of stomata and trichomes. Multicellular tector trichomes were observed on the abaxial surface of the midrib. The present study also yielded similar observations, indicating that varieties HY2 and Sirugumani possess trichomes consisting of 1-3 cells. It has been observed that vessel elements undergo the development of tracheoids and idioblasts. It is suggested that these tracheoids possess the ability to retain water. The vein endings in the leaves are connected to them. Tracheoids with similar characteristics were also identified in the current study within the HY2 variety (see Figure 2c).

The leaf surface exhibits a glossy texture and is adorned with trichomes, which serve the purpose of reflecting intense light that is incident upon it. The aqueous hypodermal layer plays a crucial role in water storage. It is noteworthy that this layer exhibits a greater thickness (two-layered) on the upper surface, as opposed to the lower surface (single-layered). The leaves of the P. betle plant contain a significant amount of mucilage and oil-secreting cells. As a result, this plant has the ability to effectively reduce heat and minimise water loss.

Petiole:

The cross-sectional view of the petiole consistently displays a convex shape on the lower side and a concave shape on the upper side, regardless of variations. The petiole consists of a single layer of epidermal cells that exhibit a slight papillose texture. The trichomes are situated directly above the uniseriate and 1 - 4 celled

epidermis. JB and KAR demonstrate a cellular range of 5 to 6 cells, as illustrated in Figure 2g. Below the epidermis, there is a discontinuous layer or band of collenchyma, usually consisting of approximately 7-8 layers, except in Sirugumani, where it consists of only 5-6 layers. The recorded quantity of girdles was found to be between 10 and 11, surpassing the previously reported figure of 8 by Chibber in 1913. The petiole's central region features a notable mucilage canal, as shown in Figure 2f. Figures 2h-2j illustrate the presence of secretory cells and calcium oxalate crystals, comprising both sand and rod-shaped crystals, distributed throughout the parenchyma region. The vascular bundles of the petiole demonstrate schizostely and are arranged in a circular pattern, as illustrated in Figure 2f. There is a total of seven large alternating bundles, each accompanied by four small bundles, except in LV where there are five large bundles alternating with five small bundles. Furthermore, there are stipular ones that last for two or three minutes, as depicted in Figure 2k. The petiole is comprised of a central mucilage canal, which is accompanied by two smaller mucilage canals on either side. Among these options, HY2 exhibits the presence of multiple mucilage canals, with a maximum of nine canals, each positioned opposite to a vascular bundle. The canals are organised in a 'U' shape, along with the existence of a prominent central canal. The ground parenchyma cells were found to contain calcium oxalate and sand crystals. The findings reported by Murthy (1973) and Raman et al. (2012) are similar in nature.

Conclusion:

The internal structure of leaves, stems, and petioles exhibited similarities in their gross internal structure, with minor observed variations. The vegetative organs are enveloped by a thin layer of cuticle. In all the varieties examined, two distinct types of trichomes were identified: unicellular and uniseriate multicellular. The hypodermis consists of a single layer of cells in the leaves. The hypodermis also exhibits secretory cells and mucilage canals. The upper epidermis lacks stomata. The lower epidermis, similar to the upper epidermis, consists of a single layer of cells. These cells are approximately the same length as the upper epidermal cells, but their width is slightly smaller, resulting in an oblong shape. The stomata exhibit a uniform distribution on the lower surface. The stomata exhibit an anomocytic type of structure. The mesophyll tissue is characterised by the presence of calcium oxalate and fine sand crystals.

Consistently, across all variations, the shape of the petiole exhibits a convex outline on the lower side and a concave outline on the upper side. Secretory cells and calcium oxalate crystals, also known as sand crystals, are distributed throughout the parenchyma region. The distinguishing characteristic of the petiole is the existence of collenchymatous girdles located beneath the epidermis. The number of layers in these girdles, as observed in the examined varieties, ranges from 5 to 8.

From a transactional perspective, the stem exhibits an outer rind region consisting of an epidermis comprised of cells with thick walls, which is further protected by a substantial cuticle layer on the outside. The cortex exhibits the presence of a collenchymatous layer, typically ranging from 2 to 8 cells in width. Additionally, oil cells can be observed within the cortex. There is a prominent mucilage canal located at the centre of the stem, measuring approximately 10 - 15 mm. Furthermore, there is a series of 7 - 8 smaller canals that are present, effectively separating the two rings of vascular bundles.

The significant occurrence of mucilage canals in the stem and petiole suggests a potential reduction in transpiration rate. Additionally, mucilage exhibits hydrophilic properties, allowing it to efficiently absorb moisture from the surrounding atmosphere and undergo complete dissolution. The presence of discontinuous collenchyma strands in the stem and petiole elucidates the supportive characteristics of this tissue in facilitating climbing and twining behaviours. The observed anatomical features of the leaf, petiole, and stem in all the studied varieties are commonly xeromorphic. These characteristics may contribute to the preservation of aroma and the extended shelf-life of P. betle leaves. A morpho-anatomical key has been developed for the varieties that were examined.

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